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Experimental animals and *in vitro* systems in the study of lymphocytic choriomeningitis virus *

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The history of lymphocytic choriomeningitis (LCM) research is reviewed from the point of view of whether the main discoveries concerning LCM pathogenesis have stemmed from animal or in vitro *research methods. Most of the results initially stemmed from animal experiments, but in recent years recourse has increasingly been made to* in vitro *techniques to confirm and amplify the animal-based conclusions.*

Different research approaches are discussed and an attempt is made to assess the role of in vitro *versus animal methods in obtaining knowledge on this virus. The same analysis is applied to the future needs and trends in arenavirus research.*

It is now generally recognized and taught (*1*) that acute lymphocytic choriomeningitis (LCM) of mice is due to a violent immune response of the animal in an attempt to rid itself of its own diseased cells. The virus is essentially harmless but the thymus-derived lymphocytes of the host react against the new viral antigens that appear in the surface membranes of infected cells.

The history of this interesting virus shows that about 15 years ago the concept was quite new that a virus could induce a lethal immune disease analogous to a generalized homograft rejection or graft-versus-host response (*2*). Since that time this phenomenon has gained wide acceptance and the key role of cellular immunity in the destruction of infected cells in many animal and human virus diseases is now recognized (*3–5*).

It is of interest to determine whether these mechanisms of virus pathogenesis could have been discovered by *in vitro* methods alone. It is most unlikely or even impossible that this could have been done. Early hints of the mechanism are available, retrospectively, in the observations of Burnet & Fenner (*6*) on Traub's work on the innocuous effects of congenitally-transmitted LCM in mouse colonies. Obviously the concept of immunological tolerance to viruses represents the converse (or complementary) statement of the virus-induced autoimmune defence reaction. Both Traub (*7, 8*) and his associate Rowe (*9*) suspected the importance of some type of " tissue immunity " in the development of disease after LCM infection. The evidence and nature of this factor were further elucidated by Hotchin & Weigand (*10*) and by Hotchin (*2, 11*). More details and elegant demonstrations of the tolerance–rejection phenomenon were further developed by Volkert (*12, 13*) and others (*14–16*).

A review of the foregoing publications up to 1970 reveals that in every case all the data were based on animal experiments alone and no conclusions were drawn from *in vitro* or tissue culture techniques. It was only after 1970 that research into LCM pathogenesis began to utilize *in vitro* methods, extensively. Further, it becomes evident that the *in vitro* methods were brought into use only as confirmatory experiments to clinch points not easily demonstrated in the animal. Even so, several of these experiments were of critical importance to the central concept of cellular immune response as a major factor of virus disease pathogenesis and recovery. Examples include: (1) the *in vitro* demonstration of T cell attack on virus infected cells; (2) the lack of a major cytopathogenic effect (CPE) due to LCM on different tissue types; (3) the development of immunofluorescent and electron microscopic labelling methods for LCM antigen location; and (4) the application of several of the foregoing to show the cyclical transcience of LCM infection at the cellular level.

The demonstration of a cellular immune response in LCM, as distinct from a lethal effect of the virus upon the infected cells, proved elusive until *in vitro* techniques were perfected. Traub (*17*) failed to demonstrate neutralizing factors in lymph node extracts from infected or LCM-immune mice,

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although slight virus-neutralizing capacity was shown by extracts of immune spleen tissue. Benson (*18*) described the first attempt to demonstrate a cytotoxic effect of LCM-immune spleen cells upon LCM-infected mouse testis monolayers *in vitro*. The destruction was seen several days after exposure of the infected cells to the immune spleen cells. Normal spleen cells caused no destruction but the immune cells caused a minor destructive effect upon normal target cells. These observations have been confirmed and extended by Lundstedt (*19*) and others (*20*) using the liberation of a radioactive label to follow cell lysis. This *in vitro* technique has been used as the basis for studies of the role of histocompatibility genes in the LCM-infected cell rejection phenomenon (*21*).

Studies of cytopathogenicity obviously require the usual *in vitro* examination. Early papers on LCM virus in tissue culture showed virus growth with slight or temporary CPE (*22*, *23*). Since then, many papers on LCM tissue culture have confirmed the early conclusions (*24*). The lack of CPE prevented the development of a useful plaque assay until a sensitive suspended BHK21/13S cell assay system was devised (*25*). The BHK21/13S plaque assay system has replaced mouse titration for most virus research work with LCM and is applicable to other arenaviruses.

The development of immunofluorescence for LCM research has parallelled its use in other virus systems. It is now used mainly with *in vitro* (tissue culture) cells as antigens for diagnostic tests for human serum antibody and has been applied to refined studies on the distribution of LCM antigen in animal tissues.

The availability of accurate quantitative *in vitro* methods of virus cultivation, coupled with serological methods for specific antigen detection, has made possible the detailed analysis of the sequences involved in virus infection and replication in the cell. These studies have demonstrated the transient, self-limiting nature of LCM infection (*26*, *27*) and have had an interesting application in the elucidation of observations made on the *in vivo* murine infection. Workers on LCM have long been perplexed by the ability of many cells to remain visibly uninfected (by immunofluorescent observation) in an animal which for weeks or months had supported peak virus titres of 10^{5}–10^{7} LD_{50}/g in all tissues and organs. This phenomenon was clearly pinpointed by Mims & Subrahmanyan (*28*) using peritoneal macrophages. The work on cyclical transient infection with LCM not only explains this effect but provides a good illustration of the valuable results obtained from the interaction of *in vivo* and *in vitro* techniques in research on virus pathogenesis.

The current trend for many papers to be published on the biochemical analyses of virus-infected cells (*29*) would not be possible without almost total reliance on *in vitro* tissue culture methods. Virtually no work at all of this type has been done using animal tissues as source material, owing to the greater biochemical complexity of this substrate and the greater difficulty of time relationships and purification procedures.

A new and undeveloped area of virological research concerns the interaction of the slow or chronic neurotropic viruses with behavioural control mechanisms of the host. Obviously this is a case where *in vitro* cell systems could be expected to be of little help. Nevertheless, a possible parallel between the two has already been revealed. In my laboratory a behavioural disturbance was found to be induced in mice persistently infected with LCM virus (*30*). More rigorous study of this model by Seegal (*31*) has confirmed the early observations and has strengthened the conclusion that this virus can cause abnormal behaviour in animals.

The parallel with *in vitro* studies has come about via chemical investigation of the brain neurotransmitters and their rate-limiting enzymes. A preliminary comparison of the brains of LCM-carrying and normal mice by Dr Fredrick Baker in this laboratory has shown that the activity of choline acetyltransferase (EC 2.3.1.6), expressed as counts per minute of ^{14}C-labelled acetylcholine synthesized per milligram of brain protein, is 8–13% higher in the virus-infected animals than in normal animals. In a similar biochemical study by Welsh & Oldstone (*32*) using normal mouse neuroblastoma cells and those persistently infected with LCM, these authors reported a 13% increase in choline acetyltransferase in the LCM-infected line compared with the controls.

This seems a rather unexpected parallel which, if substantiated, would suggest that the behavioural changes induced by LCM infection of the brain may be mediated by a rather simple derangement of cerebral neurotransmitter enzymes. Such as early solution to a subtle and difficult problem is almost too good to be true, and it may prove fallacious; nevertheless, the concept is very plausible that brain virus causes a biochemical alteration that is reflected in altered behaviour.

This concept is not in itself new. Viruses have

been blamed for almost every human ill, including mental disease, for many years. Recently, some excellent reviews have searchingly examined the real possibility that pathogenesis of some forms of schizophrenia has a slow virus etiology (*33*, *34*). It seems likely that this area may prove to be a fruitful but intensely difficult one for a new investigative approach to human mental disease. The need for animal, especially primate, research here is quite obvious. It is also clear that an *in vitro* electrophysiological study of virus-infected neuronal cells might be quite productive, particularly with viruses that do not quickly kill the cell.

An analysis of the main subdivision of LCM diagnosis and research reveals that there is still a heavy dependence on animal techniques, in spite of the applicability of most of the standard *in vitro* methods to the LCM model. Table 1 lists some of these techniques and subdivisions and shows the relative applicability of tissue culture and animal methods. While this list will vary according to definitions and work interests, it is clear that the distribution of usefulness between tissue culture and animal methods is fairly even, with a slight bias towards animal use. It would clearly be impossible to pursue much of current LCM research if animal research were to be abolished; nevertheless the literature reflects a trend over the years from almost total dependence upon animal work towards a steadily increasing proportion of tissue culture-based methods and results. In view of the enormous complexity of the *in vivo* cellular environment, this trend can be expected to continue for some time. With most animal viruses, however, the main motivating force for continued research is disease-oriented; this will exert a steady pressure towards the use of animal models. Without these, the final questions of virus-induced human disease will remain unanswered.

LCM and other arenaviruses provide an excellent example here. As the prototype virus in this increasingly important group, LCM has played a key role in uncovering the major pathogenetic mechanisms of all the arenaviruses. It is quite remarkable how the parallel mechanisms, which are apparently shared by all the members of the arenavirus group, have been repeated in Bolivian (*35*) and Argentinian (*36*) haemorrhagic fevers and in Lassa fever. The same mechanism of an immunologically tolerant "carrier state" small-animal host reservoir, with spillover to man via urine contamination, has been repeatedly observed with each of these diseases.

Table 1. An analysis of the use of tissue culture and animal methods in LCM virus research and diagnosis

Subdivision of virus diagnosis and research	Method	
	Tissue culture	Animal
Virus–cell interactions		
biochemistry of infected cells	+	
microscopy of infected cells (CPE)	+	
immunology of infected cells	+	+
Pathogenesis		
cellular immunity	+	+
humoral immunity		+
immunological tolerance		+
immunosuppressive effects		+
footpad effects		+
toxic effects	+	+
virus–tumor interactions	+	+
behavioural effects		+
Diagnostic methods and assays		
immunofluorescence	+	
complement fixation	+	
neutralization	+	+
plaque assay	+	
animal titrations		+
animal sentinels		+
Total	10	12

While the list of properties of Junin and Machupo viruses is becoming comparable to that of LCM (Table 1), progress with Lassa virus lags behind owing to its extreme virulence and danger for man (*37*, *38*). But as special isolation facilities become available and epidemiological data are gathered, all the present evidence indicates that Lassa fever (with the possible exception of its virulence) will show the same features as the other arenaviruses and will exhibit pathogenic mechanisms similar to LCM. This in turn offers hope that when useful vaccine and therapeutic breakthroughs have been developed by *in vitro* and animal techniques for LCM, the same principles and methods may well hold for Lassa fever.

Judging by the increasing international turmoil caused by the transportation of Lassa fever cases and personnel at risk, arrival of these preventive and therapeutic breakthroughs will be none too soon.

RÉSUMÉ

ANIMAUX D'EXPÉRIENCE ET SYSTÈMES *IN VITRO* POUR L'ÉTUDE DU VIRUS DE LA CHORIOMÉNINGITE LYMPHOCYTAIRE

L'historique des recherches sur la CML au cours de 15 années environ révèle que la plus grande partie des découvertes relatives à la pathogénie de cette affection ont été faites grâce à des expériences sur les animaux plutôt que par des méthodes *in vitro*. L'élaboration de la théorie d'une attaque immunitaire à médiation cellulaire sur les antigènes de membranes tissulaires induits par le virus confirme cette conclusion. Depuis 1970 environ, les méthodes de culture de tissus ont connu un emploi de plus en plus large et tendu à remplacer l'utilisation des animaux. Plusieurs des observations faites au cours de travaux sur l'animal ont été corroborées et élégamment expliquées grâce à des méthodes *in vitro*. C'est particulièrement vrai en ce qui concerne les travaux sur l'attaque immunitaire à médiation cellulaire et sur la nature transitoire de l'infection des cellules par le virus de la CML. La plupart des titrages de ce virus sont maintenant effectués par la méthode des plages qui tend à remplacer le titrage sur l'animal.

En revanche, les nouvelles études récemment entreprises sur les effets du virus de la CML sur le comportement des souris infectées ne pouvaient être faites que sur l'animal dans son intégralité, en tant que sujet. Il en est de même d'autres travaux sur les effets des virus lents qui, au stade actuel, sont fondés principalement sur des expériences chez les animaux. Il semble réellement possible que certains troubles psychiatriques humains puissent être attribués à des effets de virus lents du système nerveux; c'est pourquoi, les effets comportementaux des infections virales chroniques du cerveau vont être de plus en plus explorées et cela nécessitera des expériences sur des primates.

Le parallèle établi entre la CML et les autres maladies humaines à arénavirus, plus graves, imposera la nécessité d'expériences tant *in vitro* que sur l'animal en vue de parvenir à préparer des vaccins.

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